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INNOCUITY AND TOXICITY TESTING OF NON–VIRAL COMPONENTS OF FOOT–AND–MOUTH DISEASE VACCINES

F. Sólyom

ABSTRACT

Some biological properties of 3 different adjuvants: saponin, DEAE-dextran and sodium azide applied in foot-and-mouth vaccines have been studied. As tested in guinea pigs, by dialysis of various batches of saponin, the author succeeded in reducing their toxicity by 80 to 90%, their hemolytic activity by 60 to 75% and their inflammatory capacity by 40 to 50%, whereas in cattle the inflammatory capacity of dialyzed saponin batches decreased by 80 to 90%. The inflammatory capacity of DEAE-dextran is about 50 to 60% less than that of the saponin. The effect of saponin and sodium azide has directly been studied in virus suspension also. Neither the saponin nor the sodium azide affected adversely the infectivity and immunogenicity of the antigen. The loss of potency due to the storage of the vaccine is not in relationship with the presence of the two above-mentioned components.

The potency of food-and-mouth disease (FMD) vaccines can be considerably increased by the use of appropriate adjuvants. Out of these, saponin has greatly enhanced the immunogenicity of FMD vaccines for cattle and sheep, whereas diethyl-amino-ethyl-dextran (DEAE-dextran) and oil proved to be the adjuvants of choice in pig vaccines.

Sterility, which is an essential prerequisite of virus vaccines, has been ensured by the addition of 0.05% sodium azide (Na-azide). The immunogenicity of the FMD vaccine depends not only on the quality but also on the quantity of the adjuvant. The optimal quantitative relations cannot be, however, furnished with saponin, because various saponin preparations have untoward biological effects, causing severe tissue injury or inflammation. Dalsgaard (1972 a, b) was able to reduce the adverse effects of saponin compounds by dialysis, thereby making possible the use of greater quantities for the adjuvation of FMD vaccines.

Out of the non-viral components of FMD vaccines, saponin, DEAE-dextran, and Na-azide were tested in this laboratory for innocuity and toxicity.

MATERIAL AND METHODS

Antigens

A 10% suspension was prepared from live FMD virus propagated in bovine lingual epithelium cell culture. Aliquots of this suspension were, after the addition of different components, titrated for infectivity for the sake of comparativ evaluation. Aliquots of similar suspension were, after inactivation with 0.04% formalin for 41 h at 26°C, completed with various adjuvants, and the experimental vaccines so obtained were used for comparative antigenicity titrations.

Dialysis

20% solutions of the saponin samples were dialyzed against a 20-fold volume of distilled water in Kalle tubes, at 4°C.

RESULTS

Toxicity assays

The saponin samples were assayed for toxicity by intraperitoneal, intramuscular and subcutaneous administration to inbred Balb/c x CBA F_1-mice, weighing 20-22 g. The mean LD_{50}-values obtained in these tests with non-dialyzed and dialyzed samples of saponin are shown in Fig. 1.

Hemolytic activity

The saponin samples were tested for hemolytic activity with red blood cells of various animal species. Hemolysis was measured by spectrophotometry and the results were expressed in terms of 50% hemolysing dose (HD_{50}). The results obtained with the same samples before (Fig. 2) and after dialysis (Fig. 3) show a marked increase of the HD_{50}-values in the dialyzed lot.

Assay for phlogogenic effect

0.5% solutions of saponin and DEAE-dextran were injected in 0.05 ml dose into one hind footpad of mice, while the contralateral pad was similarly treated with an identical dose (0.05 ml) of PBS. The animals were exterminated at different points of time after treatment, the two hind feet were cut off at the tibio-tarsal joint, and each was weighed. The phlogogenic effect of the saponin samples was evaluated on the basis of the weight difference. The temporal course of the inflammatory reactions elicited by saponin and DEAE-dextran was also followed up. The results are shown in Fig. 4. The surplus weight due to inflammation is expressed in per cents related to the weight of the untreated foot as 100.

In the next experiment the phlogogenic effect of the dialyzed and non-dialyzed saponin samples was compared by injecting the pairs of them into opposite hind foot pads of the same mouse.

The mean weight data of the treated feet of 5 mice are compared in Fig. 5. The local phlogenic action of saponin was less pronounced, when, simultaneously with the injection into the foot pad, it was given to the recipient also by another route. The local anti-inflammatory action of parenteral saponin doses depended considerably on the route, as shown in Fig. 6.

The phlogogenic action of saponin was also tested on guinea pigs, which were treated intradermally with the samples. Reading of the results was easy, because the round cutaneous ulcerations, appearing at the site of injection 10 days after treatment, were large enough to express the results as the number of mm of diameter length. The mean value obtained on treatment of the guinea pigs with dialyzed and non-dialyzed samples are shown in Fig. 7.

Tests of dialyzed and non-dialyzed samples in cattle were evaluated by excision of the changed area after slaughter and comparison of the weights of the inflammatory swellings (Fig. 8).

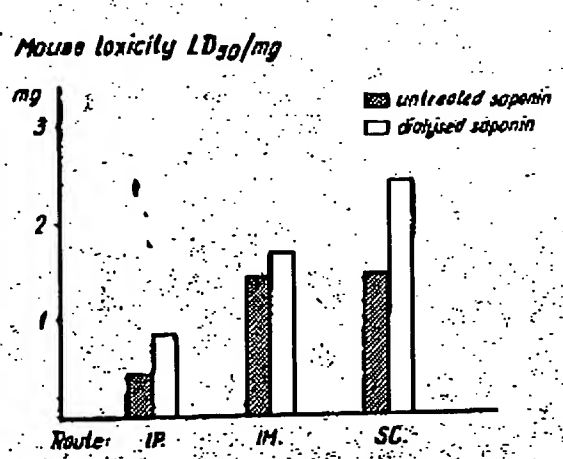

Fig. 1. Toxicity assay of untreated and dialyzed saponin in adult mice. Mean of five replica experiments.

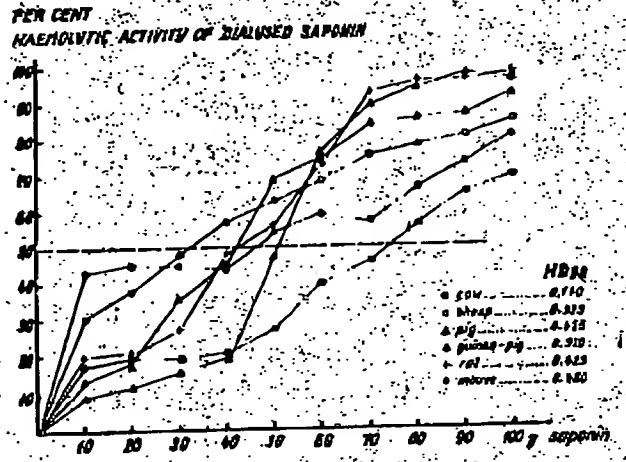

Fig. 3. Hemolytic activity of dialyzed saponin samples against red blood cells of various species.

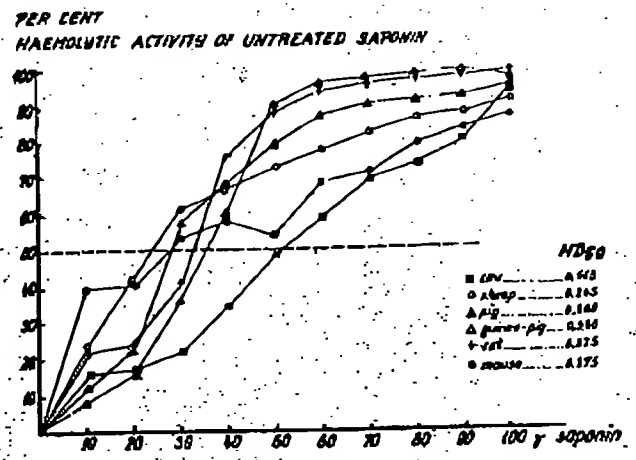

Fig. 2. Hemolytic activity of non-dialyzed saponin samples against red blood cells of various species.

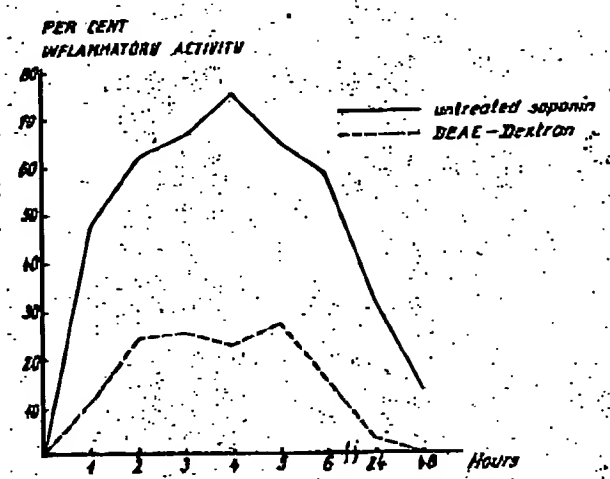

Fig. 4. Phlogogenic action of saponin and DEAE-dextran in the mouse footpad test (reaction versus time).

Effect of saponin on the infectivity of the virus

Various amounts of saponin were added to aliquots bovine lingual epithelium cell suspension containing 10% virus, which were thereafter stored at 4°C. The saponin-treated virus suspensions and an untreated suspension serving as control were titrated for infectivity in suckling mice at different points of time. The results, expressed as LD_{50} for the mouse, are shown in Fig. 9.

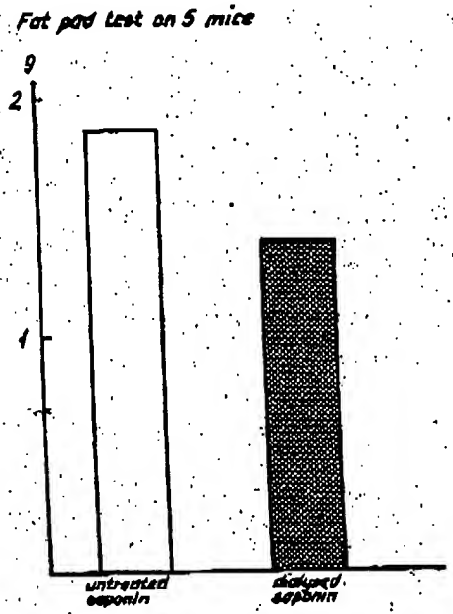

Fig. 5. Phlogogenic effect of dialyzed and non-dialyzed saponin samples in the mouse foodpad test.

Effect of saponin on the antigenicity of the vaccine

This effect was tested with three different samples of saponin in various vaccine preparations. The latter had been tested for potency in adult mice by E-index method, and in the course of the potency tests, FMD virus adapted to adult mice was titrated on vaccinated and unvaccinated mouse groups. The E-index value corresponds with the difference of the infective titres determined in the control and vaccinated groups. The vaccine was regarded as potent, if its E-index value was 2.39 or higher (3).

The effects of the three different saponin samples were compared by adding them in dialyzed and non-dialyzed forms to the same basal vaccine preparations. The results are shown in Fig. 10.

Saponin-free and saponin-adjuvanted variants of further 5 vaccine preparations were also tested for potency by the E-index method, at different points of time following finishing (Fig. 11).

Effect of sodium azide (Na-azide) on the infective titre

According to the foregoing schemes, 10% virus suspensions were titrated for infectivity at different points of time following the addition of various quantities of Na-azide (Fig. 12).

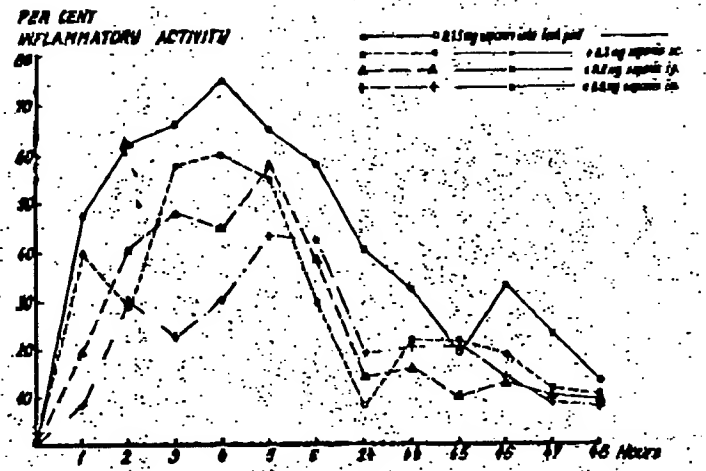

Fig. 6. Phlogogenic action of saponin on administration into footpad alone, and into footpad intraperitoneally.

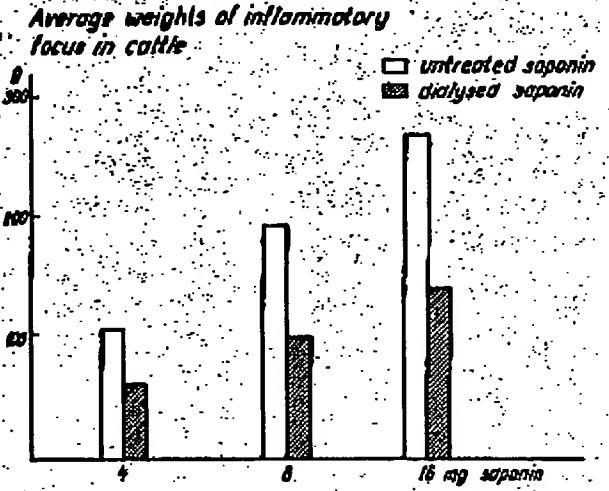

Fig. 8. Inflammatory local response of cattle to subcutaneous administration of dialyzed and non-dialyzed samples of saponin.

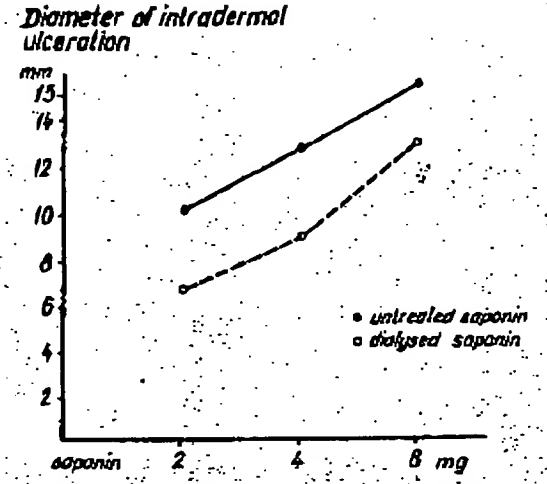

Fig. 7. Inflammatory local response of guinea pigs to intradermal administration of dialyzed and non-dialyzed samples of saponin.

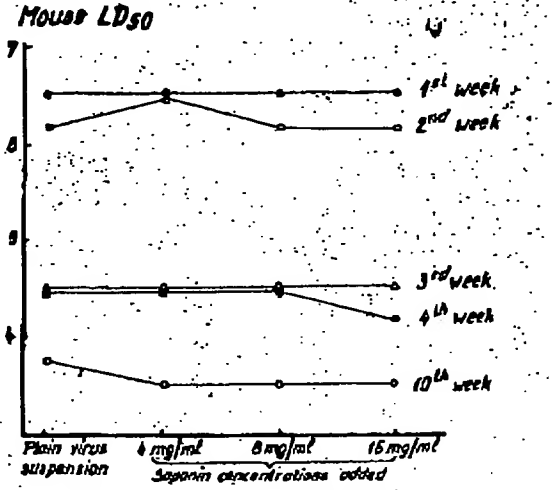

Fig. 9. Effect of saponin on the infectivity of the virus.

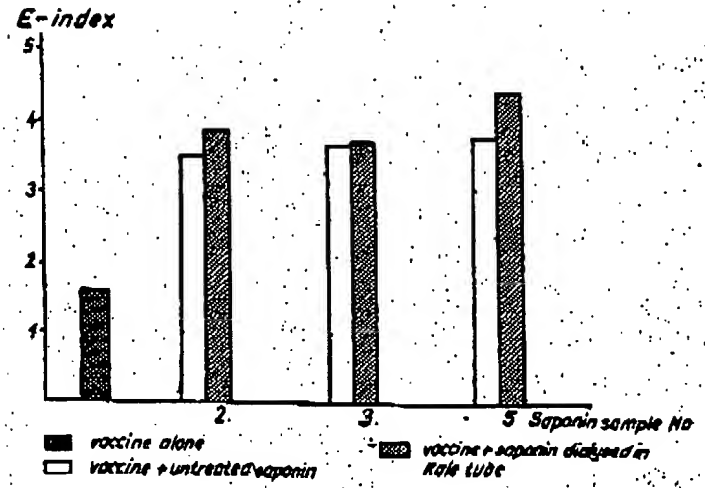

Fig. 10. The adjuvant effect of dialyzed and non-dialyzed saponin as determined with the E-index method.

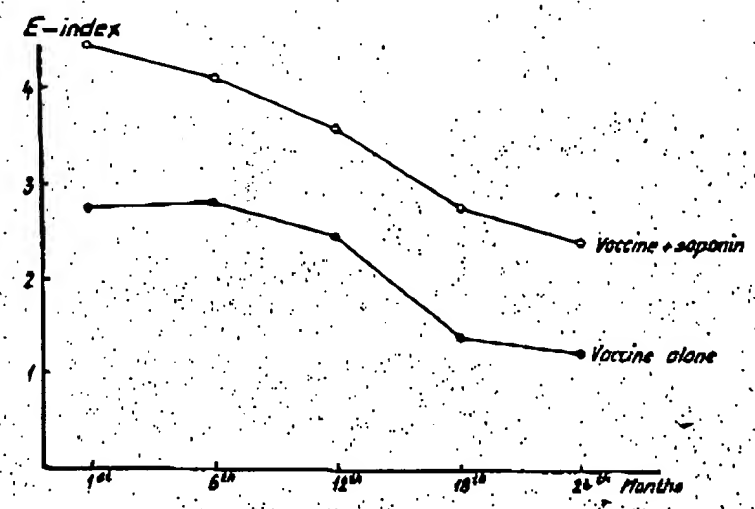

Fig. 11. Immunogenicity versus time as determined by the E-index method in FMD vaccines containing and not containing saponin.

Effect of Na-azide on vaccine potency

Several experimental vaccines were prepared and were tested for potency by the E-index method, with or without addition of 0.05% Na-azide. The results are summarized in Fig. 13.

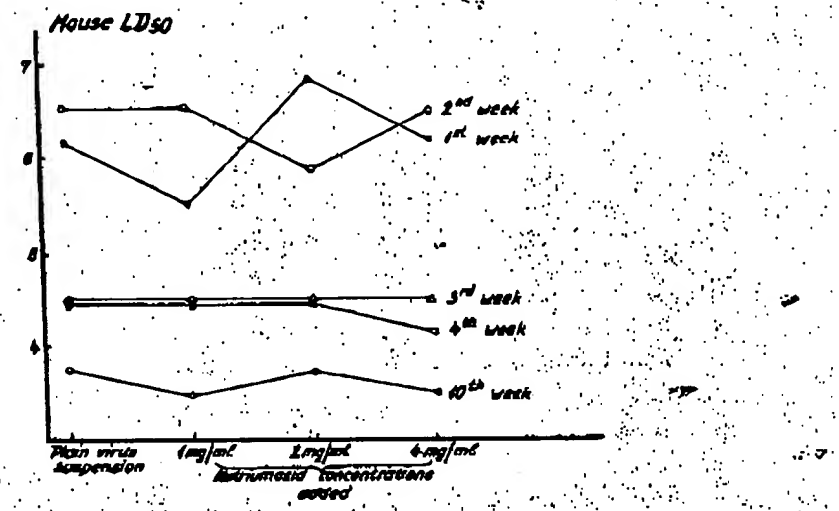

Fig. 12. Effect of Na-azide on the infective titre of FMD-virus.

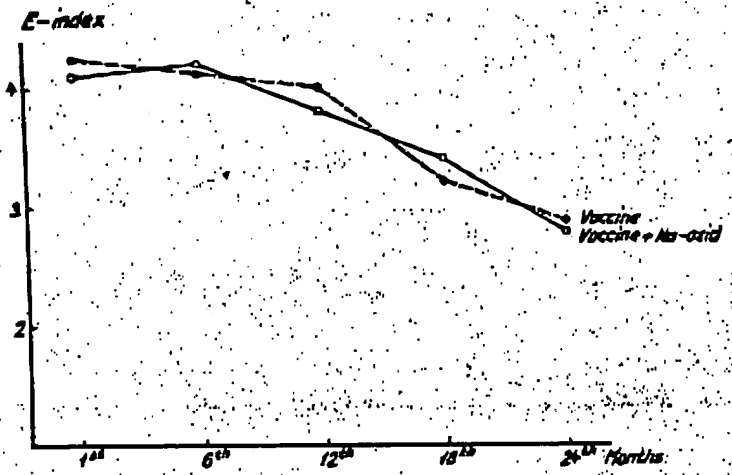

Fig. 13. Immunogenicity versus time as determined by the E-index method in FMD vaccines containing and not containing Na-azide.

DISCUSSION

Three non-viral (non-antigenic) components of FMD vaccines, i.e. saponin, DEAE-dextran and sodium azide, were tested for influence on the innocuity (toxicity) of the preparations.

Tests with dialyzed and non-dialyzed saponin samples showed that dialyzation alters certain properties of this adjuvant. The dialyzed samples were 80-90% less toxic for mice, 60-75% less hemolytic for red blood cells of various species, and proved to be 30-35, 40-50 and 80-90% less phlogogenic in mice, guinea pigs and cattle, respectively. At the same time, dialysis did not depress the adjuvant capacities of saponin, to judge from the practically identical potencies of the preparations containing dialyzed and non-dialysed adjuvant (Fig. 10). Saponin did not affect the infectivity of the virus, neither did it injure the inactivated viral antigen during long-term storage. The sam information is emerging from studies on the duration of vaccine potency (immunogenic action) (Fig. 11), which showed that the saponin-adjuvanted vaccines retained immunogenicity considerably longer than those not containing saponin. Purification of saponin by dialysis has made possible the application of greater quantities of adjuvant in FMD vaccines without increasing their adverse effect on tissues.

The phlogogenic effect of DEAE-dextran was markedly inferior to that of an identical dose of saponin. The edematous swelling induced by DEAE-dextran in the footpad of the mouse was 50-60% smaller and lasted for a much shorter time, disappearing practically within 24 h, while the saponin-induced reaction was still pronounced at that time. Saponin did not depress the infectivity of the FMD virus, to judge from the practically identical values of infectivity titres obtained at different concentrations of saponin.

Na-azide is added to FMD vaccines not so much as adjuvant as for the sake of sterility. The present experiments have shown that even 2-8 times greater quantities of Na-azide than the usual concentration failed to depress the infectivity of the virus during the period studied. Na-azide had no adverse effect on FMD vaccines on long storage either, because vaccines with and without Na-azide did not differ in potency significantly.

However, early loss of antigenicity is to be expected, if the sterility of the preparation cannot be preserved for the necessary length of time. It follows that Na-azide plays an indirect but important role in the preservation of vaccine potency.

SUMMARY

Three non-antigenic components of FMD vaccines : saponin, DEAE-dextran and Na-azide were examined for influence on vaccine quality.

It was found that certain undesirable biological effects of saponin can be advantageously reduced by dialysis. The dialyzed samples were 80-90% less toxic for mice, 60-75% less hemolytic, and 40-50% less phlogogenic than the non-dialyzed counterparties. DEAE-dextran proved to be 50-60% less phlogogenic than an identical dose of saponin.

No vaccine component tested depressed either the infectivity of the virus or the antigenicity of the inactivated FMD vaccines, whether applied at normal or at elevated concentrations. Decline of vaccine potency during storage is unrelated to the presence of any of the two examined components.

Acknowledgments

The author is indebted to Miss Zs. Sári, Mr L. Aradi and Mr L. Asztalos.

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General discussion

Chairman (P.A. Knight, UK) : I would like to observe that our experience with saponin has been that its potency as an adjuvant seems to be very much related to its inflammatory action. However, Dr Sólyom, I am not sure that you have completely demonstrated that you are not paying a price in terms of adjuvant effect when removing so much of the hemolytic and inflammatory activity.

I. Joó (Hungary) : There is no correlation between inflammatory and adjuvant activity; this fact has been confirmed several times.

J. Jacob (France) : I would like to ask a question for I am a pharmaco-toxicologist accustomed to dealing with the toxicity of drugs rather than of vaccine. I quite understand that most of the tests you utilized are what we call acute tests, but some vaccines are now beginning to be used for chronic treatment. What do you think about the usefulness of chronic toxicity test, that is, repeated doses over a more or less extended period ? Such a test could perhaps be not so complex but could nevertheless resemble the controls carried out for drugs.

Chairman : I think that the control of chronic toxicity of vaccines is a serious problem. The immunological effect of repeated administration of vaccines does not allow to make conclusions on toxicity, for reactions occurring in laboratory animals may not fairly reflect what would happen in man. On the other hand, you may have effects which remove pharmacological activities from the vaccine by specific neutralization of antibody. My own feeling is that chronic toxicity tests in the sense in which they have been applied to non-antigenic materials are not really very applicable to biologicals; I think that more emphasis could be laid upon an investigation of immunological hazards of biologicals but not in the pharmacological sense.

J. Jacob : When you have to control, for instance, a toxoid which possibly has some toxic activity, this activity may have two causes, one which is proper to the specificity of the toxin which is the origin of the toxoid; the other cause may be due to the pharmaco-toxicological properties. How can we judge the chronic toxicity of *C. parvum* vaccine intended for prolonged treatment ?

Chairman : I think that in the case of *C. parvum* vaccine we are speaking of a new category of substances. I would agree that certain of its pharmaco-dynamic effects do appear to be modified during chronic treatment. Nowadays we are applying, in this case and with a great deal of caution, certain tests of chronic toxicity.

I. Joó : Dr Hnight, may I ask you if you have any experience of testing the toxicity of bacterial vaccines in mice using anti-metabolizers, actinomycin D for instance ?

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PART VII

TOXICITY TEST SYSTEMS

Chairmen : J. Cameron (Toronto)
H.J. Ronneberger (Marburg/Lahn)

Chairman : No, I am afraid I haven't. I think that is a gap in my knowledge which needs to be rectified, but at the moment I know very little.

J. Jacob : I would like to underline the importance of chronic toxicity tests. It is very likely that in the field of sera and vaccines we have not yet attacked this problem systematically. Is not the definition of chronic toxicity also rather elastic ? In fact, we speak of "chronic" toxicity although the preparation under study may be applied for only one week or one month or as long as six months. I am sure that my colleagues the microbiologists and the immunologists will give their attention to this problem.